3025-Pos Board B180
Phase Separated Domains are Weakly Coupled to the Support in Agarose Supported Bilayers with Ternary Lipid Compositions
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Giant unilamellar vesicles (GUVs) containing two coexisting liquid phases are widely used to study lateral heterogeneity of membrane components. Previously, we reported a novel technique for depositing lipid bilayers onto agarose cushions that conserves many properties of phase separated domains in vesicles, including transition temperatures and macroscopic circular domains. In this poster, we describe our efforts to further characterize and refine these supported membranes. For example, we find that agarose supported membranes with a continuous liquid-disordered phase are more easily assembled compared to membranes with a continuous liquid-ordered phase. We also find that larger and more mobile domains are found after extended equilibration times when membranes are supported on lower melting temperature agarose compared to higher melting temperature agarose. Also, we have quantified the dynamics of domain coarsening in membranes at multiple compositions and temperatures.

3026-Pos Board B181
Effect of Sphingomyelinase Activity on the Miscibility Phase Transition in Plasma Membrane Vesicles
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The conversion of sphingomyelin into ceramide by sphingomyelinase is an early step in apoptotisis. Past experiments have shown that ceramide displaces cholesterol from liquid-ordered domains and promotes the formation of gel domains in purified vesicles [1,2]. In this work, we investigate how pretreatment of cells with sphingomyelinase alters the phase behavior of giant plasma membrane vesicles (GPMVs) isolated from these cells. GPMVs isolated from untreated RBL-2H3 cells undergo a miscibility transition near room temperature, below which vesicles contain roughly equal surface fractions of coexisting liquid-ordered and liquid-disordered phases. Untreated GPMVs also undergo micron-sized critical fluctuations within 0.5C of their transition temperature. We observe lower miscibility transition temperatures in GPMVs pretreated with sphingomyelinase in agreement with results in model membranes. We also observe an increased surface fraction of the liquid-disordered phase and more rigid ordered domains, consistent with the presence of a gel phase. GPMVs prepared from sphingomyelinase treated cells may have altered composition beyond reduced sphingomyelin and increased ceramide, and this is an area of future study. One possible implication of our current findings is that sphingomyelinase activity leads to reduced plasma membrane heterogeneity at physiological temperatures by modulating plasma membrane lipids away from a critical composition.
1. Megha and London, Ceramide selectively displaces cholesterol from ordered lipid domains (rafts): implications for lipid raft structure and function. (2004) J Biol Chem. 279, 9997.
2. Sot, Ibarguren, Busto, Montes, Goñi, and Alonso. (2008). Cholesterol displacement by ceramide in sphingomyelin-containing liquid-ordered domains, and generation of gel regions in giant lipidic vesicles. FEBS Lett, 582, 3230.

3027-Pos Board B182
Investigating Liquid and Solid Nanodomains in Model Cell Membranes using SANS
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In the past twenty years, the understanding of the cell membrane has undergone some significant changes; though previously seen as a heterogeneous mix of lipids and proteins, it is now believed to consist of distinct coexisting (though perhaps dynamic) membrane nano-environments which are key for many biological processes. Composition-controlled model membranes are ideal platforms to understand the underlying mechanisms that drive, on the one hand, phase separation and on the other, local composition and size. We have studied the binary mixtures DPPC:DOPC and DPPC:DLPC and their behavior upon the addition of an equimolar amount of cholesterol. The binary mixtures show similar gel-liquid coexistence behavior as studied by fluorescence microscopy. However, when studying these same systems using 100nmto 30nm vesicles, their temperature behavior is very different. The DPPC:DLPC system shows the break-up of domains while the DPPC:DOPC does not. Upon addition of cholesterol, DPPC:DOPC:Cholesterol shows the formation of liquid-liquid domains, as observed by fluorescence microscopy, while the DPPC:DLPC:Chol system shows the disappearance of all domains. Recent work using FRET reported that the latter system has domains, but these are in the nanometer range [Schick, Phys Rev E, 2012]. using SANS and nanometer-sized vesicles of these ternary lipid mixtures, we find that DPPC:DOPC:Cholesterol forms liquid-liquid coexisting domains while the DPPC: DLPC:Cholesterol shows no domains, in contrast to the FRET results. The role of cholesterol driving these distinct structural changes will be discussed.

3028-Pos Board B183
Influence of Calcium on Lipid Domain Formation in Agarose Supported Lipid Bilayers
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Cell membranes are complex entities whose local structure modulates important biological functions. Because of their complexity, it is difficult to observe a simple cause-and-effect relationship regarding just one aspect of cell membrane. using artificial systems, only an intended aspect of cell membrane, such as the formation of cholesterol-stabilized nanodomains can be captured in a significantly simpler system. Recently, it was shown that agarose supported lipid bilayers are decoupled sufficiently from the glass support to allow the formation of cholesterol stabilized nanodomains. In cells, these domains are too small to be directly observed. Hence, we study them using bimFCS, a camera based FCS technique, which measures diffusion of membrane markers over many length scales simultaneously. By tracing fluorescently labeled lipids such as PIP2 and Cholesterol, which interact with domains, bimFCS measures changes in the interaction of the tracers and the domains. This provides information on the stability and the size of the domains. We use the agarose supported model system in conjunction with bimFCS to study the effect of calcium ions on PIP2 containing cholesterol-stabilized nanodomains. It has been proposed that the divalent calcium ions may cross-link the negatively charged head groups and stabilize the domains. We measure the interaction of calcium with PIP2 in simple SOPC bilayers, and study more complex systems of DOPC/SM/Chol/PIP2 to model the effects of PIP2 onto cholesterol-stabilized nanodomains. We perform bimFCS measurements before and after the addition of physiologically relevant concentrations of calcium from 0.1μM to 1.8mM to study the dynamic changes of the domains.

3029-Pos Board B184
Influence of Calcium Concentration on Lipid Domains in the Inner and Outer Leaflets of the Plasma Membrane
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Many cellular functions are modulated by the spatial organization of membrane lipids. The effects of head-group-mediated lipid interactions and cytosolic factors play an important role for the lateral organization of lipids, but is less well understood than the role of the lipid fatty acid tails. The interaction of the lipid headgroups can be modulated by divalent ions. It has been reported that divalent calcium ions stabilize domains in monolayers containing phosphatidylinositol 4,5-bisphosphate (PIP2). However, it is unclear how the local concentration of calcium affects the formation of lipid rafts in the much more complex, PIP2 containing plasma membrane (PM). Here, we study the influence of calcium concentration on domain formation in PM sheets created from intact cells by sonication. We analyze the diffusion of GFP-tagged membrane proteins, which interact with the domains, using bimFCS, which measures diffusion on multiple length scales simultaneously. PIP2 is a cytosolic leaflet lipid with negatively charged head groups. To study the aggregation of PIP2 in the PM, we use GPI-GFP to study the outer leaflet of the PM, and Lyn-GFP and fluorescent PIP2 for the inner leaflet of the PM. Our results that these marker proteins reside longer in the PIP2 lipid domains at 2mM of divalent calcium ions, while this residence time is reduced rapidly as we decrease the calcium concentration. Theses results suggest a concentration dependence of calcium-induced aggregation of PIP2 in the PM at calcium levels, which may be reached in intact cells locally by opening of ion channels.